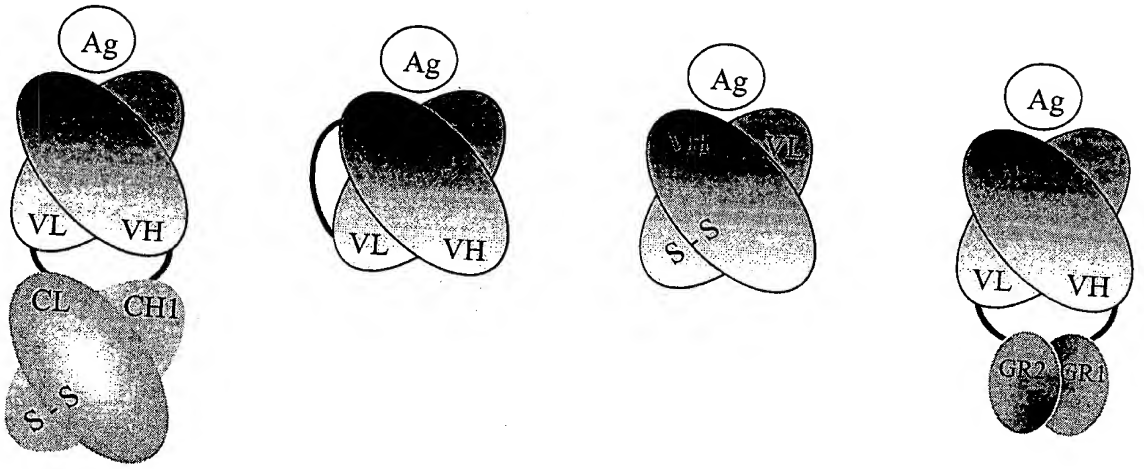
Antigen-binding units



Fab

scFv
single-chain Fv

dsFv
disulfide- stabilized Fv

ccFv
heterodimeric coiled-coil stabilized Fv

Ag antigen

Fig. 1

Sequences of coiled-coil domain in ccFv

GR1 Sequence Range: 1 to 146

```
XbaI     10         20         30         40         50
TCTAGAGGTGGAGGAGGTGAGGAGAAGTCCCGGCTGTTGGAGAAGGAGAA
 S  R  G  G  G  G  E  E  K  S  R  L  L  E  K  E  N
         60         70         80         90        100
CCGTGAACTGGAAAAGATCATTGCTGAGAAAGAGGAGCGTGTCTCTGAAC
  R  E  L  E  K  I  I  A  E  K  E  E  R  V  S  E
       110        120        130        140  AscI
TGCGCCATCAACTCCAGTCTGTAGGAGGTTGTTAATAGGGCGCGCC
L  R  H  Q  L  Q  S  V  G  G  C  *  *
```

GR2 Sequence Range: 1 to 140

```
 XhoI    10         20         30         40         50
TCTCGAGGAGGTGGTGGAACATCCCGCCTGGAGGGCCTACAGTCAGAAAA
 S  R  G  G  G  G  T  S  R  L  E  G  L  Q  S  E  N
         60         70         80         90        100
CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGG
  H  R  L  R  M  K  I  T  E  L  D  K  D  L  E  E
       110        120        130  NotI 140
TCACCATGCAGCTGCAGGACGTCGGAGGTTGCGCGGCCGC
V  T  M  Q  L  Q  D  V  G  G  C  A  A  A
```

Fig. 2

Vectors for antibody expression

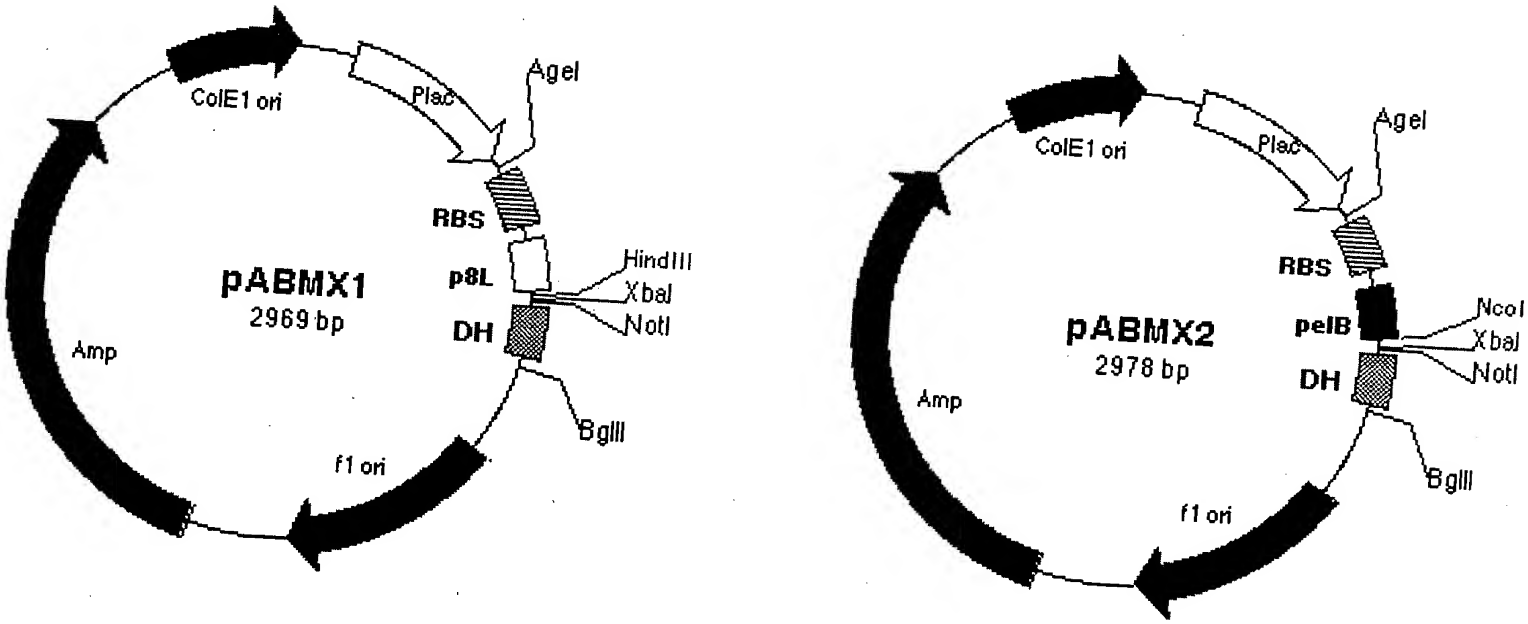


Fig. 3A

PABMX1 vector: sequence from AgeI to BglII

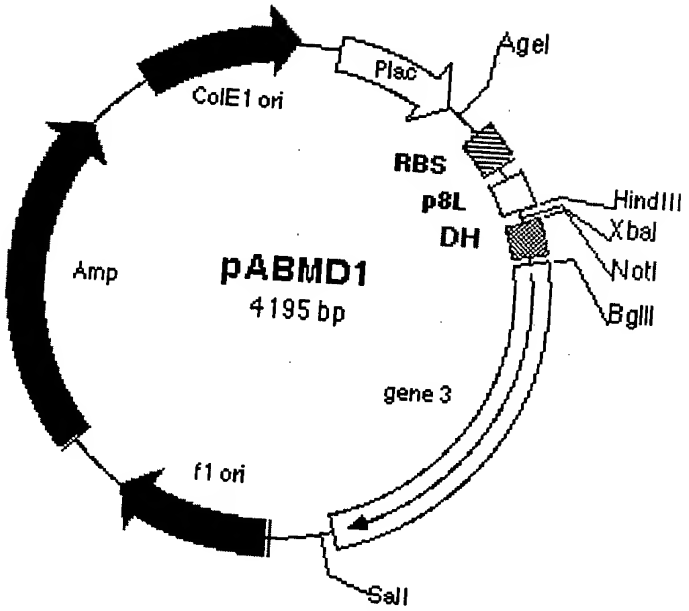
```
     lac promoter/lac O1          AgeI                EP          S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA
               P8 Leader                                                HindIII      XbaI
ATGAAAAAGTCTTTAGTCCTCAAAGCCTCCGTAGCCGTTGCTACCCTCGTTCCGATGCTAAGCTTCGCT TCTAGA
 M  K  K  S  L  V  L  K  A  S  V  A  V  A  T  L  V  P  M  L  S  F  A   S  R
    NotI                      HA-tag                      His-tag              BglII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
 A  A  A   Y  P  Y  D  V  P  D  Y  A   G  G   H  H  H  H  H  H   *   R  S
```

PABMX2 vector: sequence from AgeI to BglII

```
     lac promoter/lac O1          AgeI                EP          S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA
               pelB Leader                                    Nco I        PstI      XbaI
ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCGGCCCTGCAGGCCTCTAGA
 M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L  A  A  Q  P  A  M  A  A  L  Q  A  S  R
    NotI                      HA-tag                      His-tag              BglII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT
 A  A  A   Y  P  Y  D  V  P  D  Y  A   G  G   H  H  H  H  H  H   *   R  S
```

Fig. 3B

Vectors for antibody display



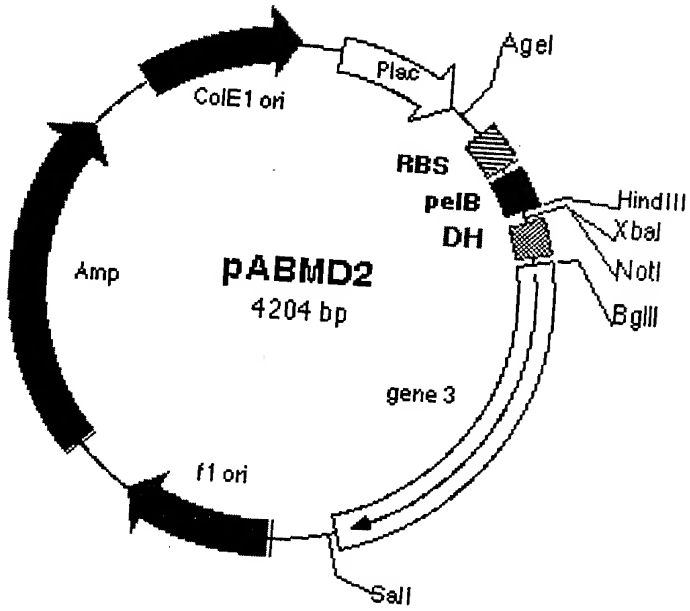


Fig. 4A

PABMD1 vector: sequence from AgeI to SalI

```
    lac promoter/lac O1          AgeI                EP        S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA
              P8 Leader                                                HindIII      XbaI
ATGAAAAAGTCTTTAGTCCTCAAAGCCTCCGTAGCCGTTGCTACCCTCGTTCCGATGCTAAGCTTCGCT TCTAGA
 M  K  K  S  L  V  L  K  A  S  V  A  V  A  T  L  V  P  M  L  S  F  A   S  R
   NotI                  HA-tag                                 His-tag       Amber stop  BglII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT  TAG    AGATCT
 A  A  A   Y  P  Y  D  V  P  D  Y  A   G  G   H  H  H  H  H  H    *      R  S
                          Gene 3                                                SalI
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
 G  G  G   T  V  E  S  C  L  A  K  ----  A  N  I  L  R  N  K  E  S  *
```

PABMD2 vector: sequence from AgeI to SalI

```
    lac promoter/lac O1          AgeI           EP        S/D
AATTGTGAGCGGATAACAATTT ACCGGT TCTT TTAACTTTAG TAAGGAGG AATTAAAAA
              pelB Leader                                          Nco I       PstI      XbaI
ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCGGCCCTGCAGGCCTCTAGA
 M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L  A  A  Q  P  A  M  A  A  L  Q  A  S  R
   NotI                  HA-tag                            His-tag       Amber stop  BglII
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT  TAG    AGATCT
 A  A  A   Y  P  Y  D  V  P  D  Y  A   G  G   H  H  H  H  H  H    *      R  S
                          Gene 3                                          SalI
GGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC
 G  G  G   T  V  E  S  C  L  A  K  ----  A  N  I  L  R  N  K  E  S  *
```

Fig. 4B

Vectors for ccFv expression

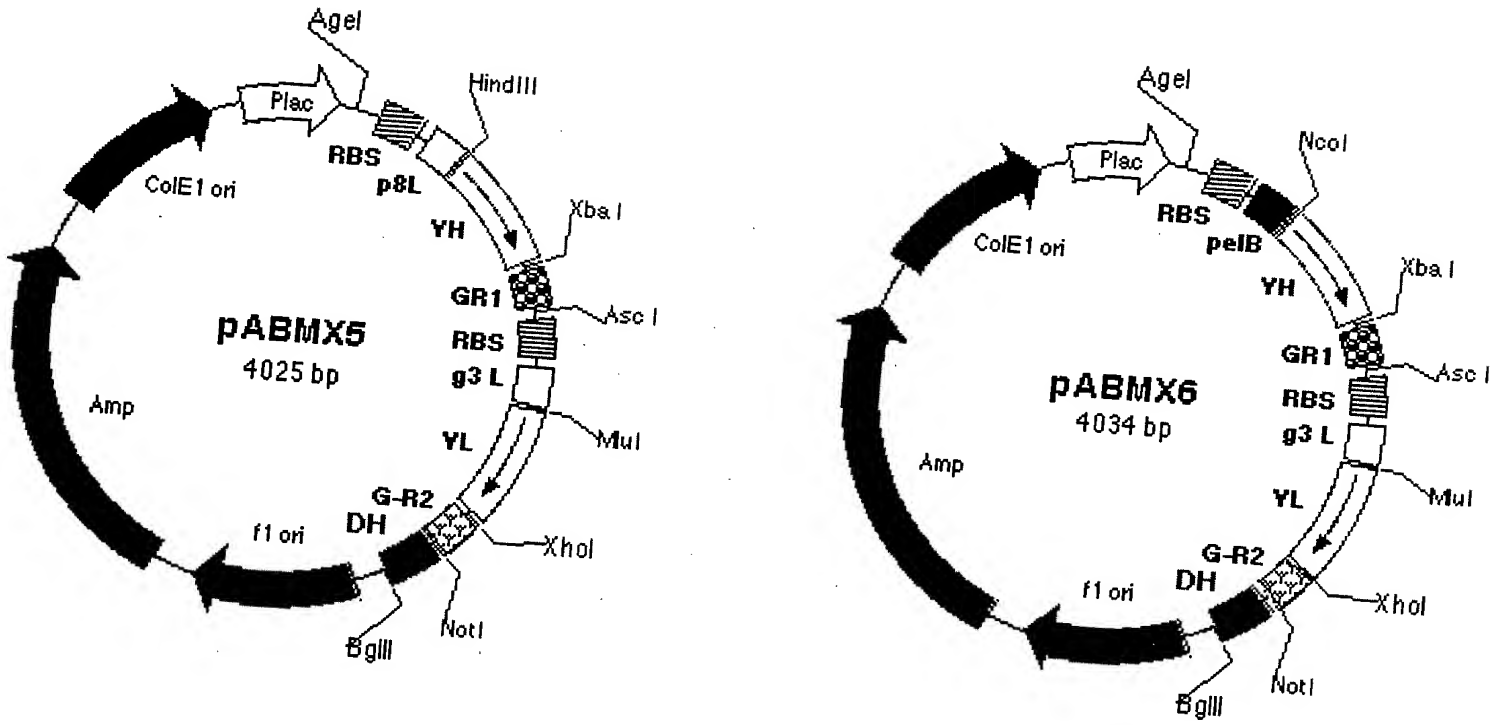
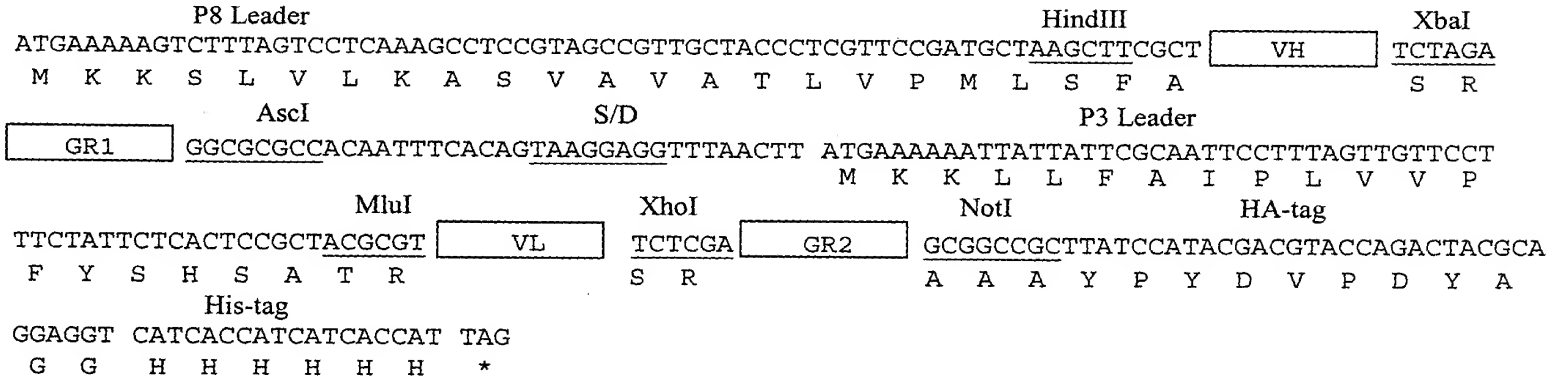


Fig. 5A

PABMX5 vector: sequence from p8 leader to DH-tag



PABMX6 vector: sequence from pelB leader to DH-tag

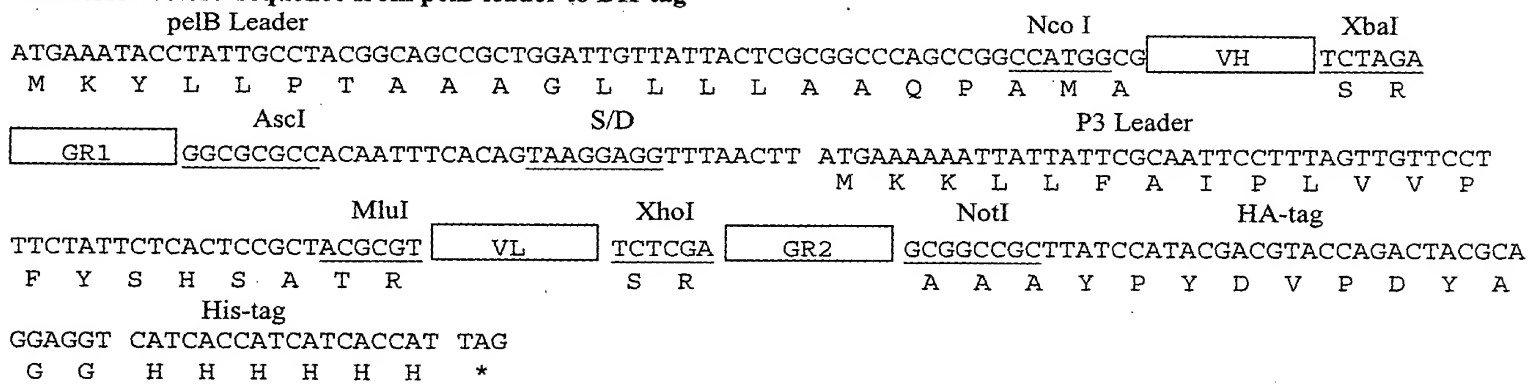


Fig. 5B

Vectors for ccFv display

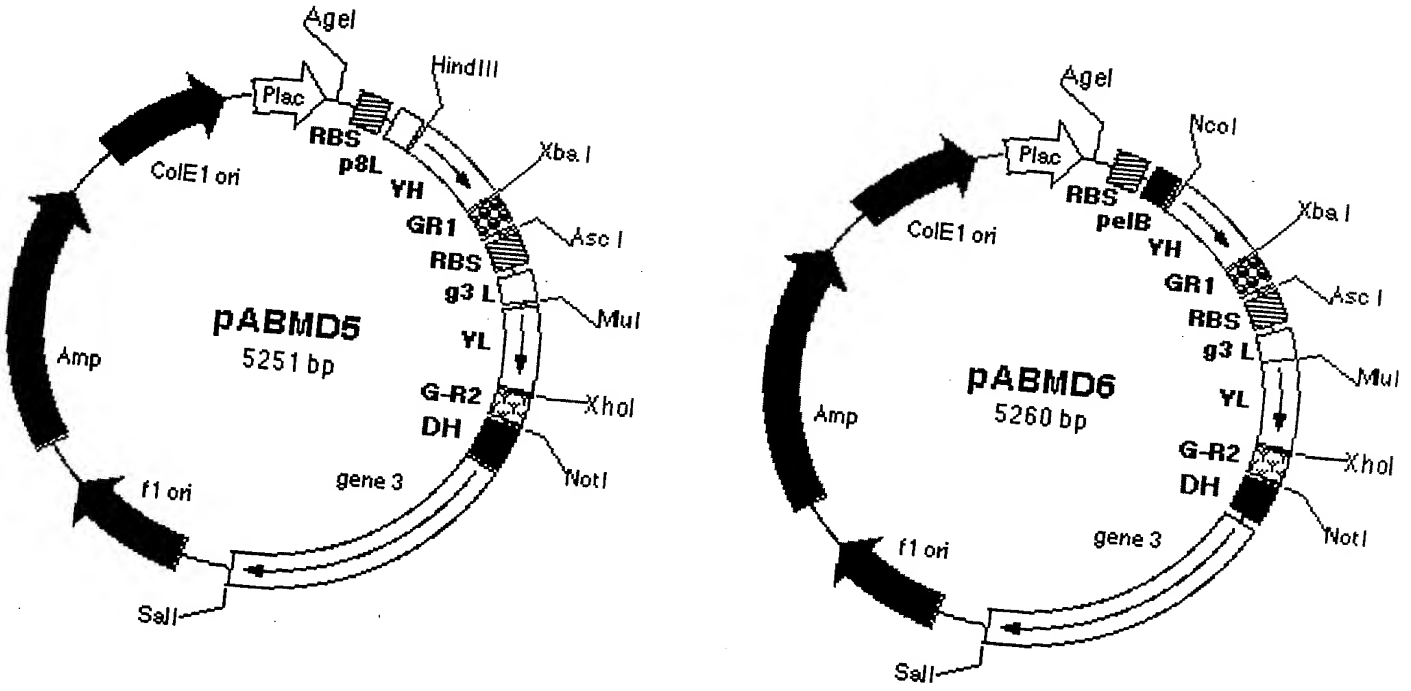
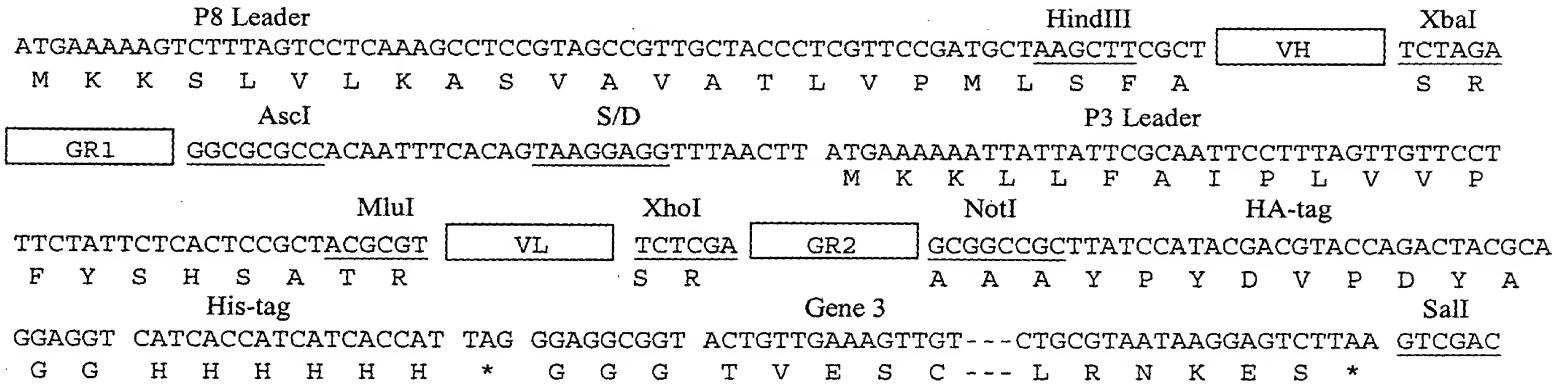


Fig. 6A

PABMD5 vector: sequence from HindIII to SalI



PABMX6 vector: sequence from pelB leader to DH-tag

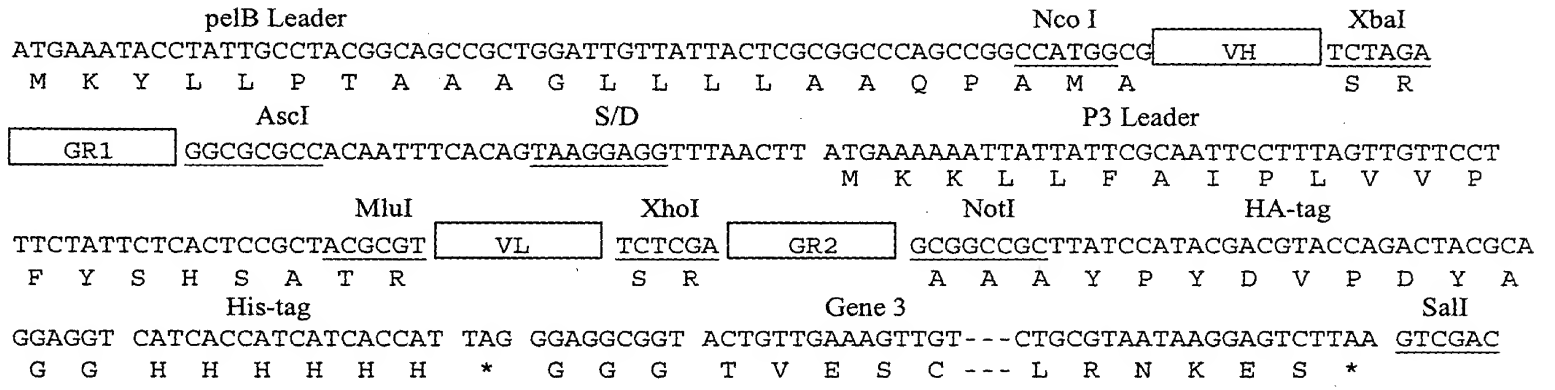


Fig. 6B

Vector for ccFv expression in Yeast

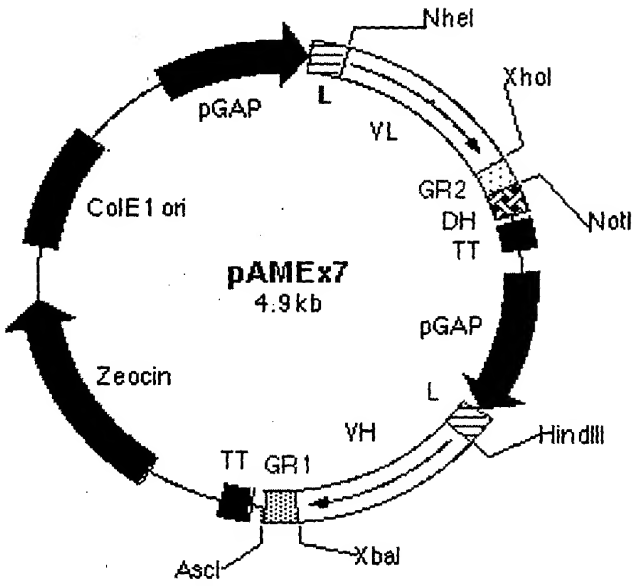


Fig. 7

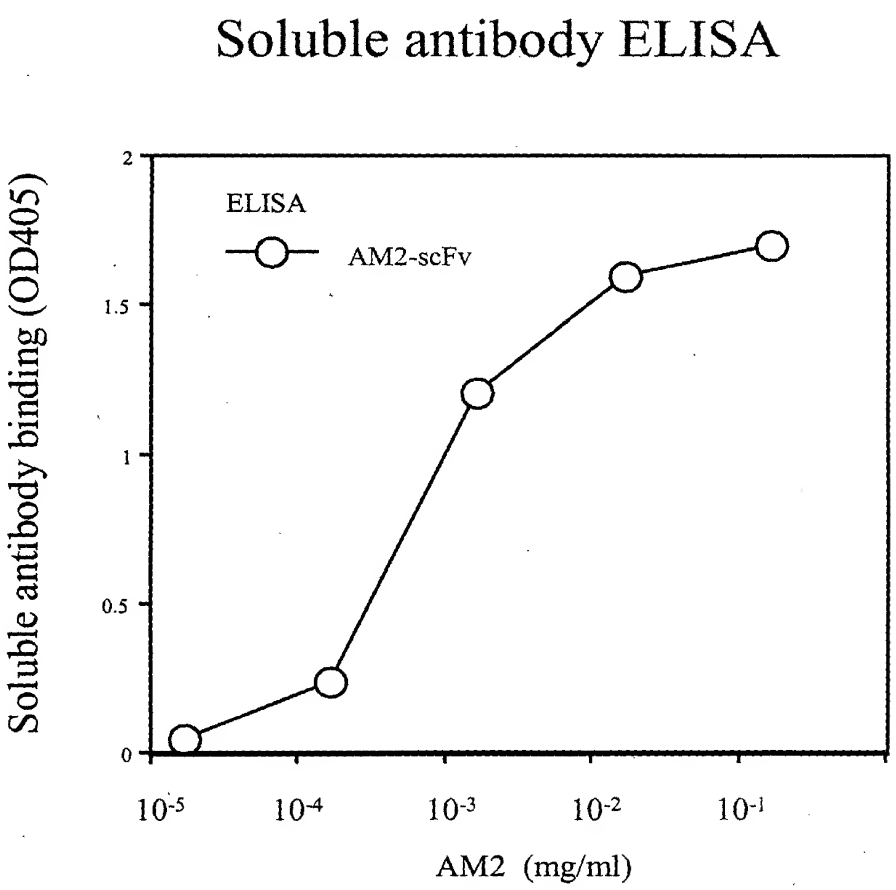


Fig. 8

AM2-scFv display on phage by pABMD1 vector

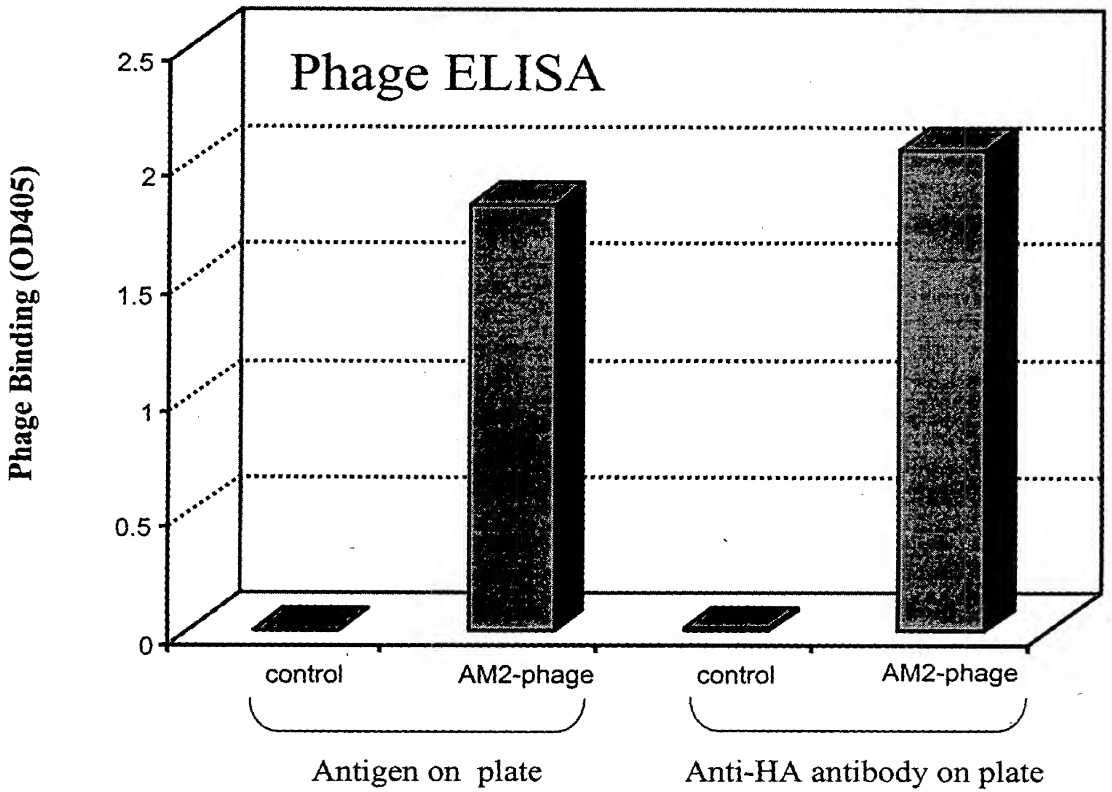


Fig. 9

Soluble AM1-ccFv antibody expression in E. coli

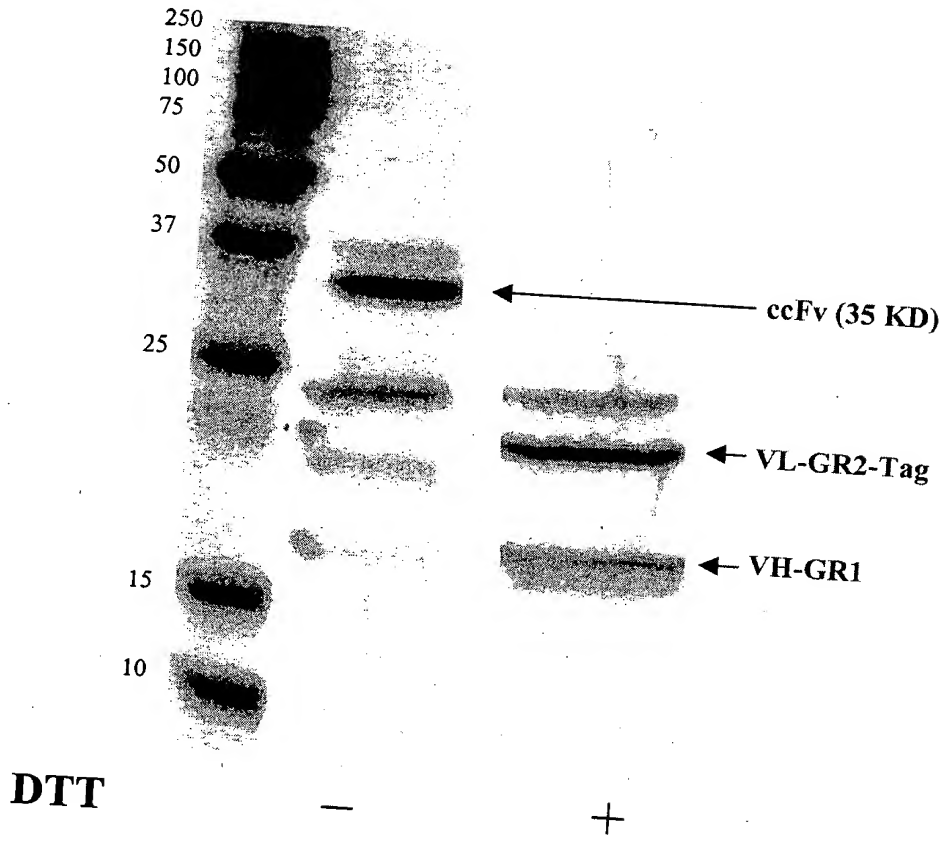


Fig. 10A

Soluble AM1-ccFv antibody binds to antigen

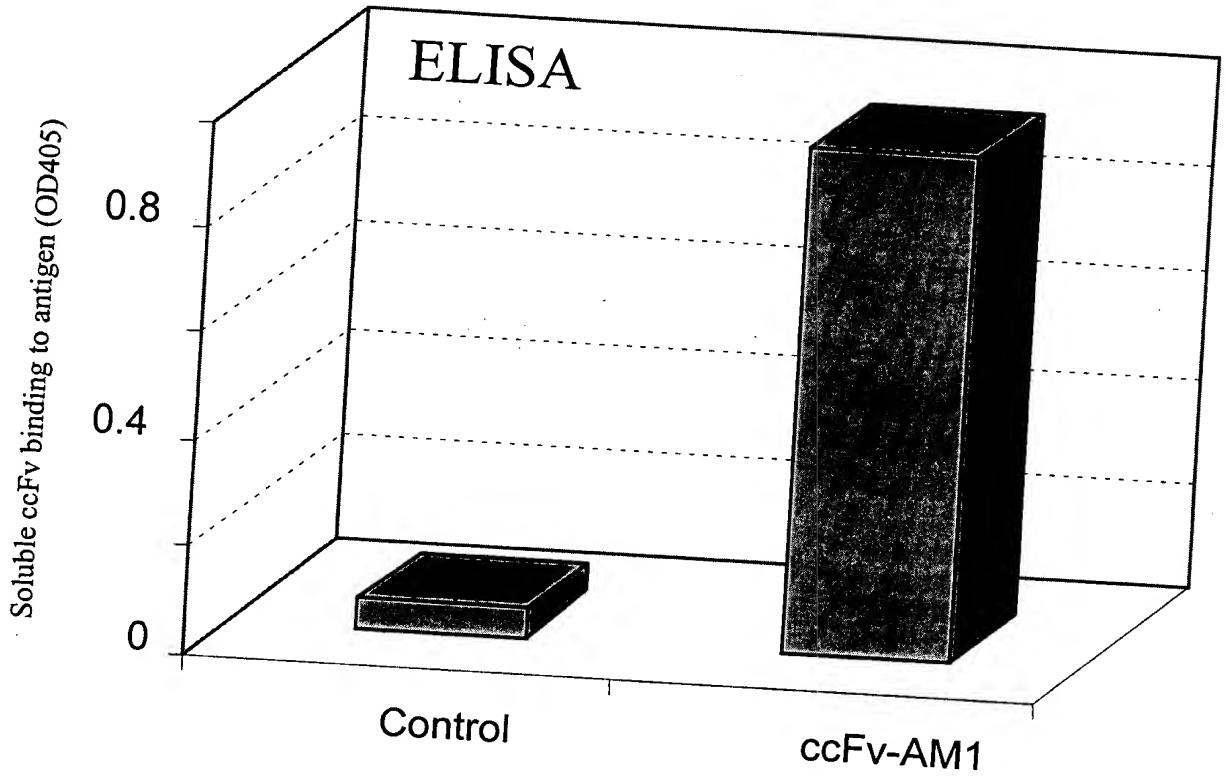


Fig. 10B

AM1-ccFv Antibody display on phage

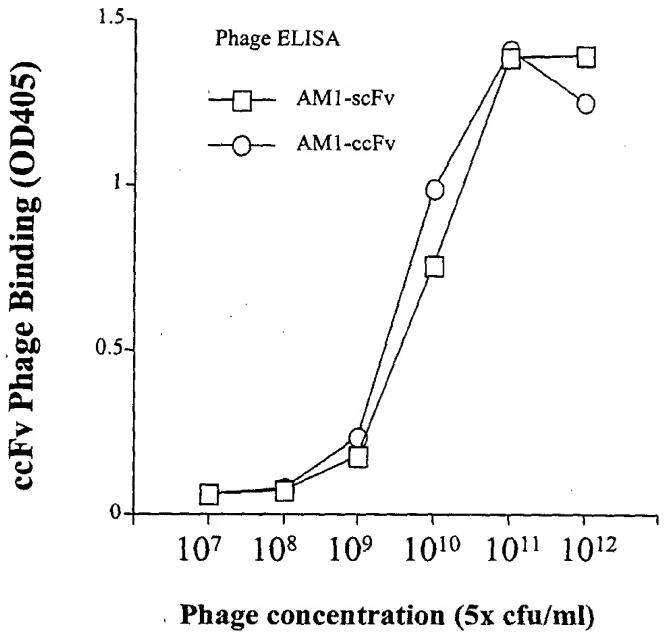


Fig. 11A

Comparison of antigen binding capability of AM2-ccFv and AM2-scFv displayed on phage particles

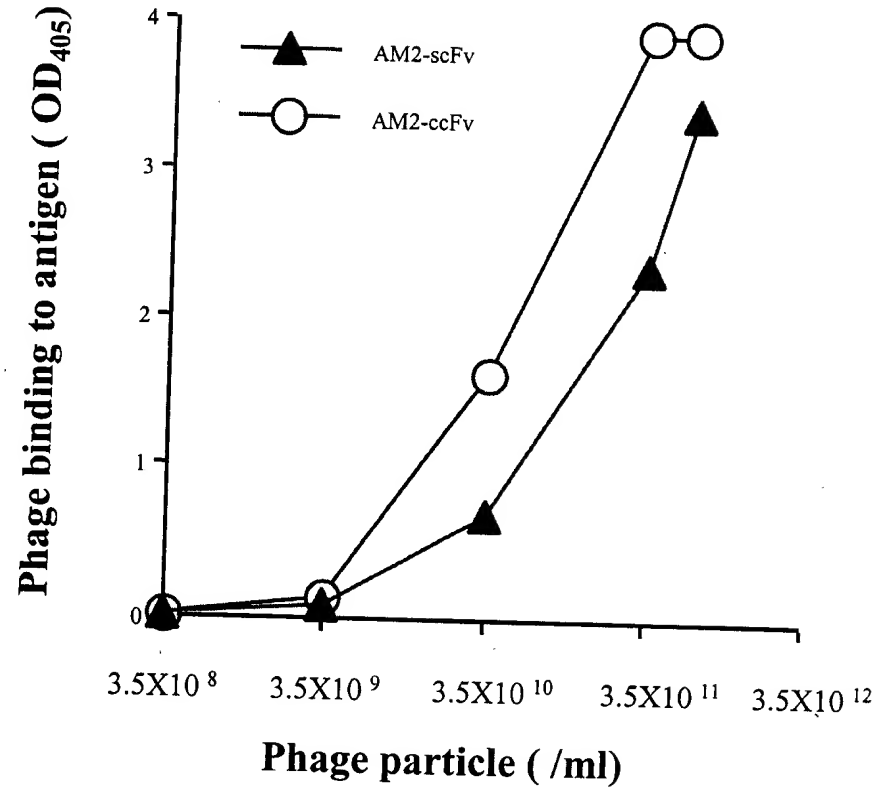
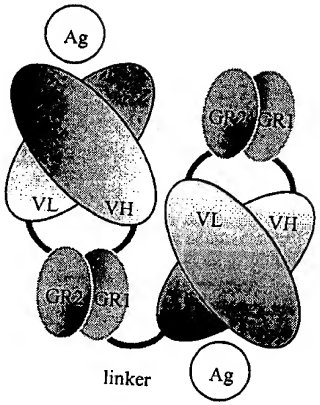
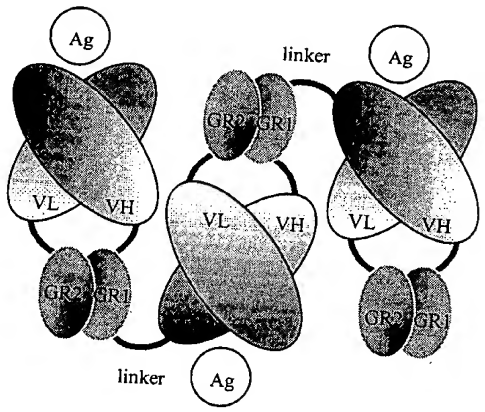


Fig. 11B

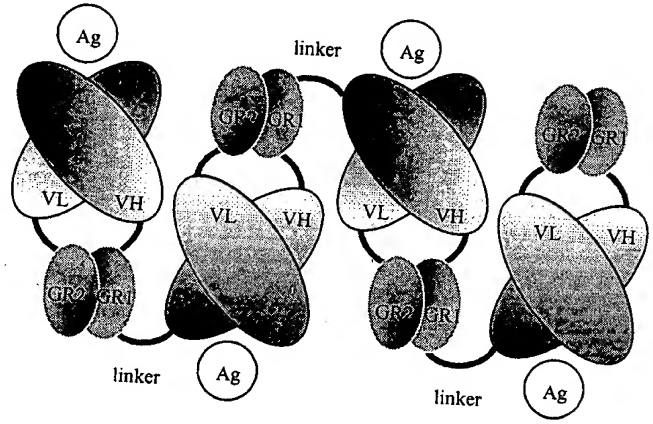
Multi-valent ccFv antibody



biovalent (ccFv)2 peptide 1: VH-GR1-VH-GR1
peptide 2: VL-GR2



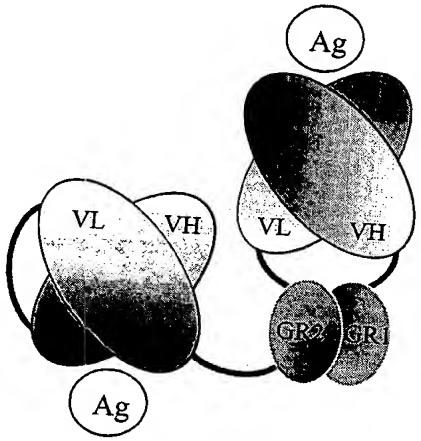
trivalent (ccFv)3 peptide 1: VH-GR1-VH-GR1-VH-GR1
peptide 2: VL-GR2



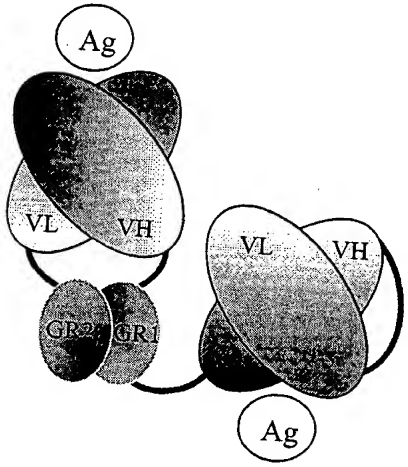
Tetravalent (ccFv)4
peptide 1: VH-GR1-VH-GR1-VH-GR1-VH-GR1
peptide 2: VL-GR2

Fig. 12

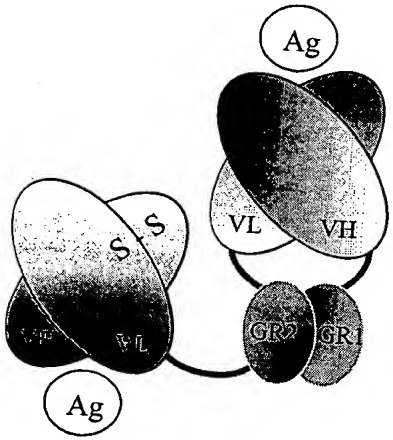
Bi-valent ccFv-scFv/dsFv antibody



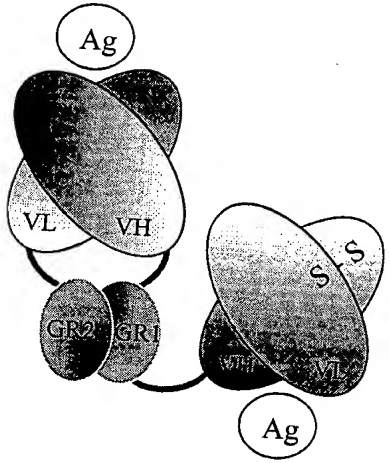
ccFv-scFv
Peptide 1: VL-GR2-scFv
Peptide 2: VH-GR1



ccFv-scFv
Peptide 1: VH-GR1-scFv
Peptide 2: VL-GR2



ccFv-dsFv
Peptide 1: VL-GR2-VL-s
Peptide 2: VH-GR1
Peptide 3: VH-s

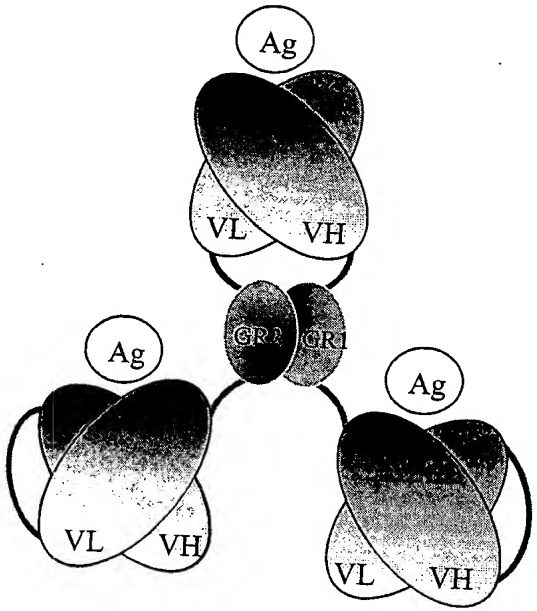


ccFv-dsFv
Peptide 1: VH-GR1-VH-s
Peptide 2: VL-GR2
Peptide 3: VL-s

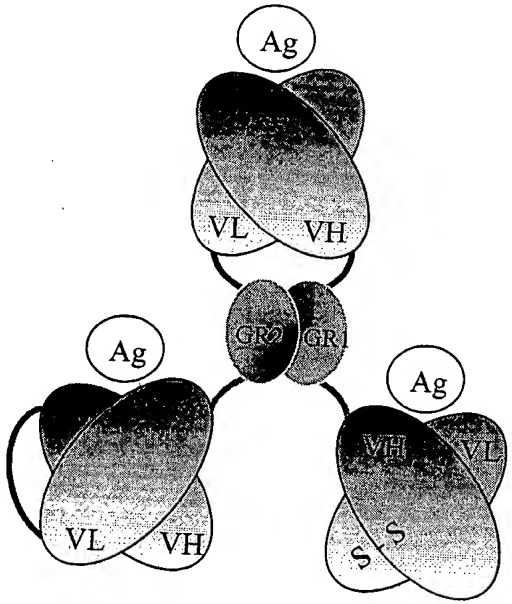
Fig. 13

ccFv-dsFv

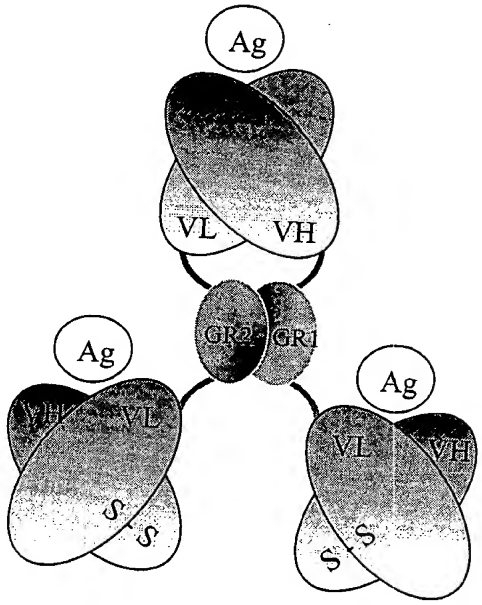
Tri-valent ccFv-scFv/dsFv antibody



Tri-valent ccFv-(scFv)2
Peptide 1: VH-GR1-scFv
Peptide 2: VL-GR2-scFv



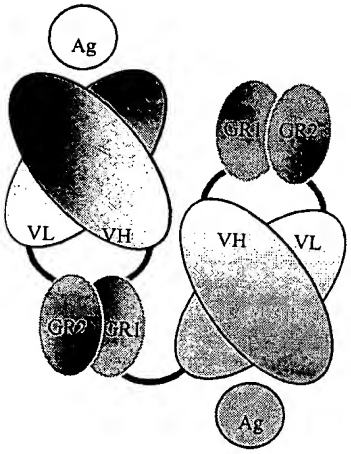
Tri-valent ccFv-scFv-dsFv
Peptide 1: VL-GR2-scFv
Peptide 2: VH-GR1-VH-s
peptide 3: VL-s



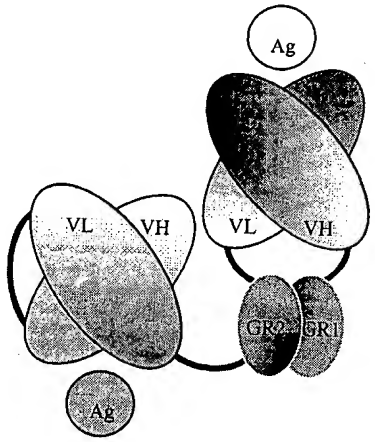
Tri-valent ccFv-(dsFv)2
Peptide 1: VL-GR2-VL-s
Peptide 2: VH-GR1-VL-s
peptide 3: VH-s

Fig. 14

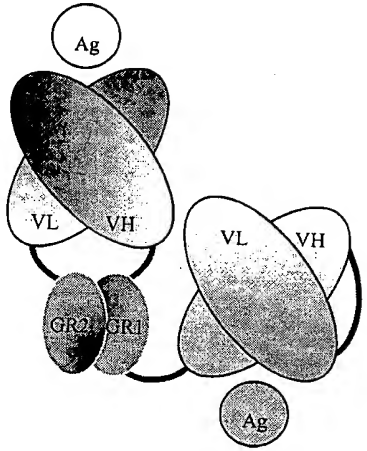
Bi-specific antibody (1)



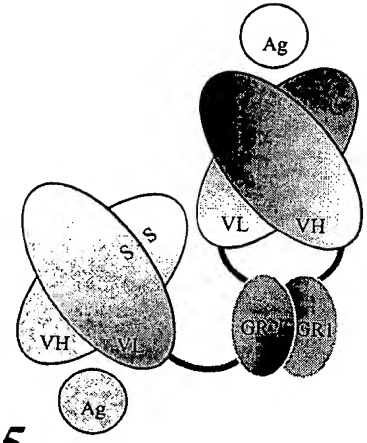
Bi-specific ccFv-ccFv'
Peptide 1: VH-GR1-VL'-GR2
Peptide 2: VL-GR2
Peptide 3: VH'-GR1



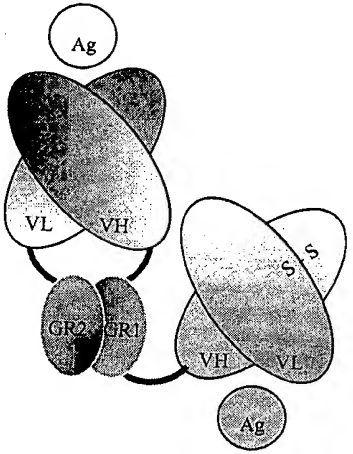
Bi-specific ccFv-scFv'
Peptide 1: VL-GR2-scFv'
Peptide 2: VH-GR1



Bi-specific ccFv-scFv'
Peptide 1: VH-GR1-scFv'
Peptide 2: VL-GR2



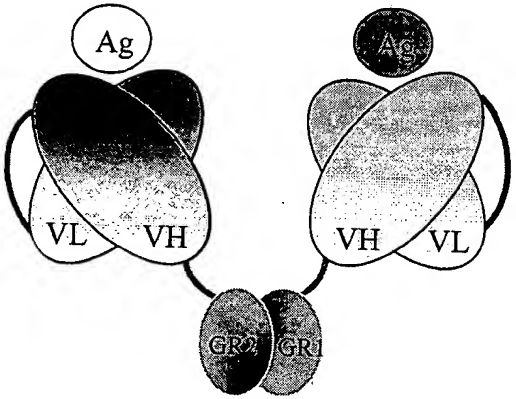
Bi-specific ccFv-dsFv'
Peptide 1: VL-GR2-VL'-s
Peptide 2: VH-GR1
Peptide 3; VH'-s



Bi-specific ccFv-dsFv'
Peptide 1: VH-GR1-VH'-s
Peptide 2: VL-GR2
Peptide 3; VL'-s

Fig. 15

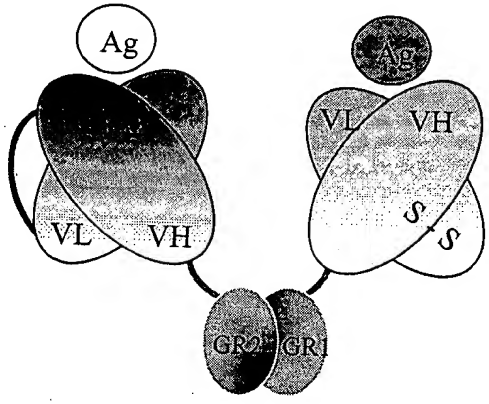
Bi-specific antibody (2)



Bi-specific scFv-ccFv'

Peptide 1: scFv-GR2

Peptide 2: scFv'-GR1

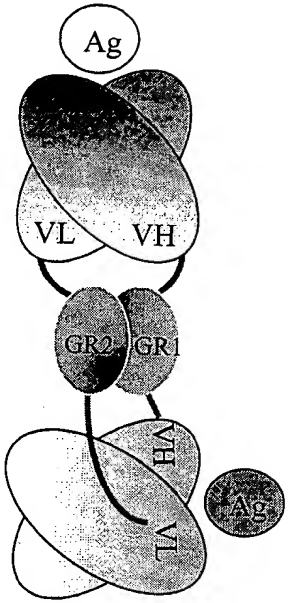


Bi-specific scFv-dcFv'

Peptide 1: scFv-GR2

Peptide 2: VH'-s--GR1

Peptide 3: VL'-s



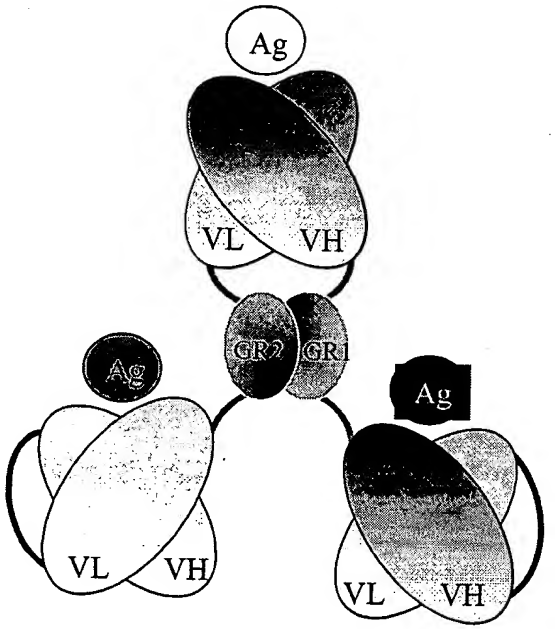
Bi-specific ccFv-ccFv'

Peptide 1: VH-GR1-VH'

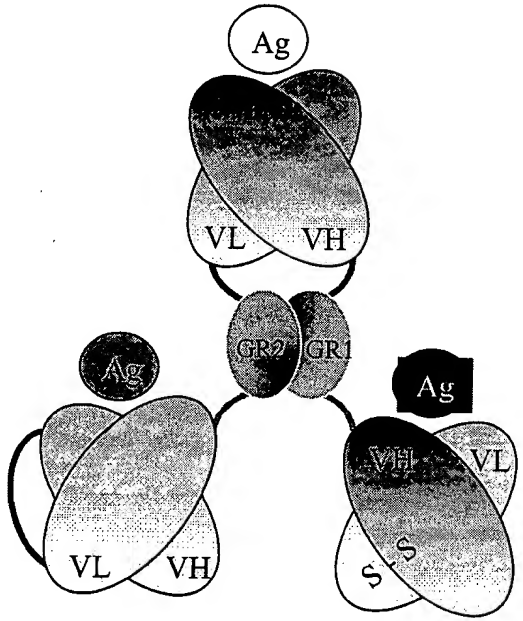
Peptide 2: VL-GR2-VL'

Fig. 16

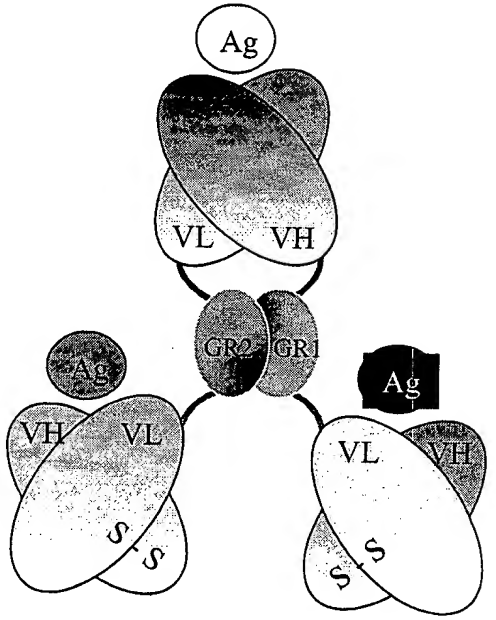
Tri-specific antibody



Tri-specific ccFv-scFv'-scFv*
Peptide 1: VH-GR1-scFv'
Peptide 2: VL-GR2-scFv*



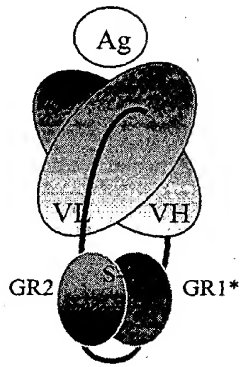
Tri-specific ccFv-scFv'-dsFv*
Peptide 1: VL-GR2-scFv'
Peptide 2: VH-GR1-VH*-s
peptide 3: VL*-s



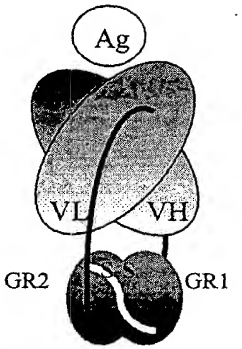
Tri-specific ccFv-dsFv'-dsFv*
Peptide 1: VL-GR2-VL'-s
Peptide 2: VH-GR1-VL*-s
peptide 3: VH'-s
peptide 4: VH*-s

Fig. 17

Single-chain ccFv antibody



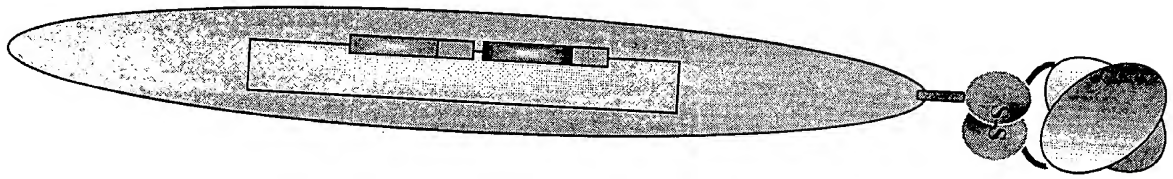
Anti-parallel coiled-coil heterodimer connected by a linker



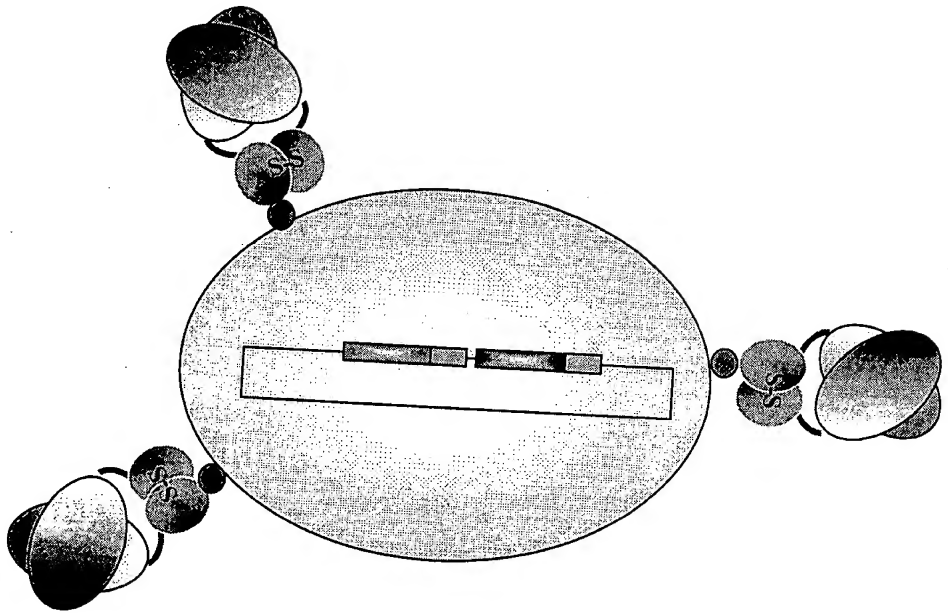
parallel coiled-coil heterodimer connected by a linker

Fig. 18

ccFv display and its use in antibody library construction



ccFv displayed on phage via coat proteins such as p3, p8, p6, p7, & p9



ccFv displayed on the surface of prokaryotic cells such as E coli; or eukaryotic cells such as yeast.

Fig. 19